Tropical Bryology 6:161-168, 1992

Phenological observations and sex ratios in *Marchantia chenopoda* L. (Hepaticae: Marchantiaceae)

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Abstract. Sex ratios were determined for 15 populations of *Marchantia chenopoda* L. along Puerto Rico. Sex was determined for twenty randomly selected individuals from each population. A female bias was observed in most populations. A census of reproductive structures of *M. chenopoda* was performed every two weeks in order to determine phenology. The highest archegoniophore count was observed to occur in January and February; minimum and maximum fertilization distances were 0.7 cm and 65 cm, respectively. Young sporophytes were most frequently observed at the beginning of the rainy season, in April and May. The highest frequency of mature sporophytes occurred during the dry season, in January and February. Capsules opened mostly during the heavy rainy season, from August to November. Protonemata were observed in the periphery of the population. Most young sporophytes aborted at the end of the moderate to heavy rainy season, from June to August. Immature gemmae cups were most frequent at the beginning of the rainy season, in April and May. Mature gemmae cups were observed throughout the rainy season, but were most frequent in September.

Introduction

Bryophytes are the second most diverse group of land plants, with approximately 14,000 species of mosses (Watson 1971) and some 6,000 species of hepatics (Schuster 1966). Nevertheless, many aspects of their biology remain relatively unknown. Aspects of their reproductive biology have been studied (Longton 1976; Mishler 1988), including gamete dispersal (Reynolds 1980; Wyatt 1977; Clayton-Greene et al. 1977), sex ratios and frequency of fertilization (Riemann 1972; Stark 1983), life history (Miles & Longton, 1987; Forman 1965) and phenology (Johnsen 1969). In contrast to the frequent reports on the phenology of vascular plants, literature on the phenology of mosses is limited, and it is even more so for hepatics.

Bischler (1984), using herbarium specimens and her collections of *Marchantia*, found archegoniophores, antheridiophores and gemmae cups present throughout the year, with a peak production of antheridiophores in August. She concluded that the initiation of reproductive structures in *Marchantia chenopoda* L._probably varies depending on local climatic conditions in the tropics and subtropics (Bischler, 1984).

This study presents sex ratios in natural populations of *M. chenopoda* and phenological observations based on stages of development in its life cycle throughout the year.

Material and Method

Sex ratios. Fifteen populations of *M. chenopoda* were surveyed along the Cordillera Central and Sierrra de Luquillo in Puerto Rico, above 480 m of elevation. Twenty individuals were randomly selected from each population. Sex was determined for each individual according to sexual reproductive structures present.

Phenology. **Site of study**. Research was conducted in the Maricao Commonwealth Forest in western Puerto Rico. The forest has a mean annual precipitation of 2577 mm (Figure 1). The mean annual temperature reported for the forest is 21.1°C (DRN, 1976). The population of *M. chenopoda* studied is located on a west-facing slope at an elevation of 780 m in the Tetas de Cerro Gordo sector. It was growing on a humid, shady road bank of lateritic clay over serpentine, within Holdridge's Lower Montane Wet Forest life zone (DRN 1976) forming a large, continuous mat of approximate 1.5 m high and 6 m wide. The population was intermixed with the hepatic *Pallavicinia lyelli* (Hook.) S. Gray and the moss *Thuidium urceolatum* Lor. Vascular associates included the ferns *Nephrolepis rivularis* (Vah) Mett., *Adiantum pyramidale* (L.) Willd., *Blechnum occidentale* L., and the angiosperms *Pilea semidentata* (Juss.) Wedd., *Clidemia hirta* (L.) D. Don, *Lobelia assurgens* var. *portoricensis* (A. DC.) Urban, and *Calycogonium krugii* Cogn.

Phenology. Sampling. Ten randomly selected permanent plots (10 cm^2) were censused every two weeks from June 1988 to July 1989. Reproductive structures representing various stages of development of archegoniophores and gemmae cups of *M. chenopoda* were collected and dissected to construct a system of classification that would facilitate the accumulation of phenological data. Since only 12 antheridiophores were observed in the population, data regarding the male structures were not analyzed.

The archegoniophore stages, **a** to **e**, are classified and described below:

a) Receptacles with archegonia. Different stages of development of archegonia are present, with the youngest archegonia near the stalk and the oldest toward the periphery. Distinctive characteristics in the field are: lobes of the receptacle not fully extended, receptacle and stalk green in color, size of whole archegoniophore approximately 1-5 mm. Archegoniophore appearance is "button-like," appressed to the thallus. This category is analogous to stage 1 of Zehr (1979).

b) Carpocephala with young sporophytes. There are young sporophytes under the carpocephalum. Field characteristics are: lobes extended, carpocephalum and stalk green in color, size of archegoniophore approximately 6-20 mm, capsules swollen and greenish. This category is analogous to stages 2 and 3 of Zehr (1979).

c) Carpocephala with mature sporophytes. There are mature sporophytes present under the carpocephalum. Field characteristics are: lobes fully extended, carpocephalum green or yellowish brown in color, size of archegoniophore approximately 21-35 mm, capsules closed, swollen and yellow. This category is analogous to stages 4 and 5 of Zehr (1979).

d) Sporophytes with open capsules. Capsules are slit open, exposing the yellowish brown spores. Field characteristics are: size of whole archegoniophore 30-35 mm, carpocephalum and/or stalk brown in color, and yellow mass of spores outside the capsule. This category is analogous to stage 6 of Zehr (1979).

e) Aborted. Abortion may occur in any of the stages **a**-**c**. Field characteristics are those of these stages, but the archegoniophore is brown in color.

The gemmae cup stages are described below:

a) Green gemmae cups. Cups and discoid propagules are green in color.

b) Dry gemmae cups. Cups are dry and brown but the propagules are green in color. They still function in dispersal.

Table 1. Percentages of female, male and sterile individuals for 15 populations of *Marchantia chenopoda* in Puerto Rico. Deviation from a 1:1 sex ratio was evaluated with a χ^2 test: ns = not significant ($P > 0.05$), * = significant ($P < 0.05$), ** = highly significant ($P < 0.01$), *** = very highly significant ($P < 0.001$).

Population	Female	Male	Sterile	
1	75	0	25	***
2	40	0	60	***
3	45	0	55	***
4	60	30	10	***
5	5	20	75	***
6	40	50	10	ns
7	25	40	35	*
8	30	55	15	**
9	85	0	15	***
10	30	5	65	***
11	55	15	30	***
12	74	0	26	***
13	10	20	70	***
14	0	59	41	***
15	20	0	80	***
Overall	40	19	41	***

Results and discussion

Marchantia chenopoda is a colonizing species. According to Bischler (1984) it produces sexual and asexual reproductive structures throughout the year, but their initiation probably depends on local climatic conditions. She examined 634 specimens, of which she reported that 40% were male and 70% were female. Fifteen hazaphard selected populations of *M. chenopoda* were sampled at random across Puerto Rico, and in the majority of them a male deficiency was observed (Table 1). Chi-square tests were performed to test deviations from the expected 1:1 sex ratio (Table 1). Forty percent were females, 19% were males, and 41% were sterile individuals at the moment of collection (Table 1). Explanations of these unequal sex ratios could be that antheridiophores may be ephemeral or shorter lived than archegoniophores, or that they were sterile at the time of collection.

Production of receptacles with archegonia (stage a) was observed throughout the year (Fig. 2a), but the major production occurred during the dry season (Fig. 1), from January to February. Regression analysis between rainfall and production of receptacles with archegonia was significant ($r^2 = 0.42$, $P < 0.05$). During this season there is water available for fertilization, since it rains almost every afternoon of the year, but there is not so much water as to wash away the antherozoids from the population. Only 12 antheridiophores were observed in the population; they were surrounded by female plants forming patches of sporophytes.

The frequency of fertilization indicated by the production of sporophytes was very high in the patch, although sporophytes were scattered throug-

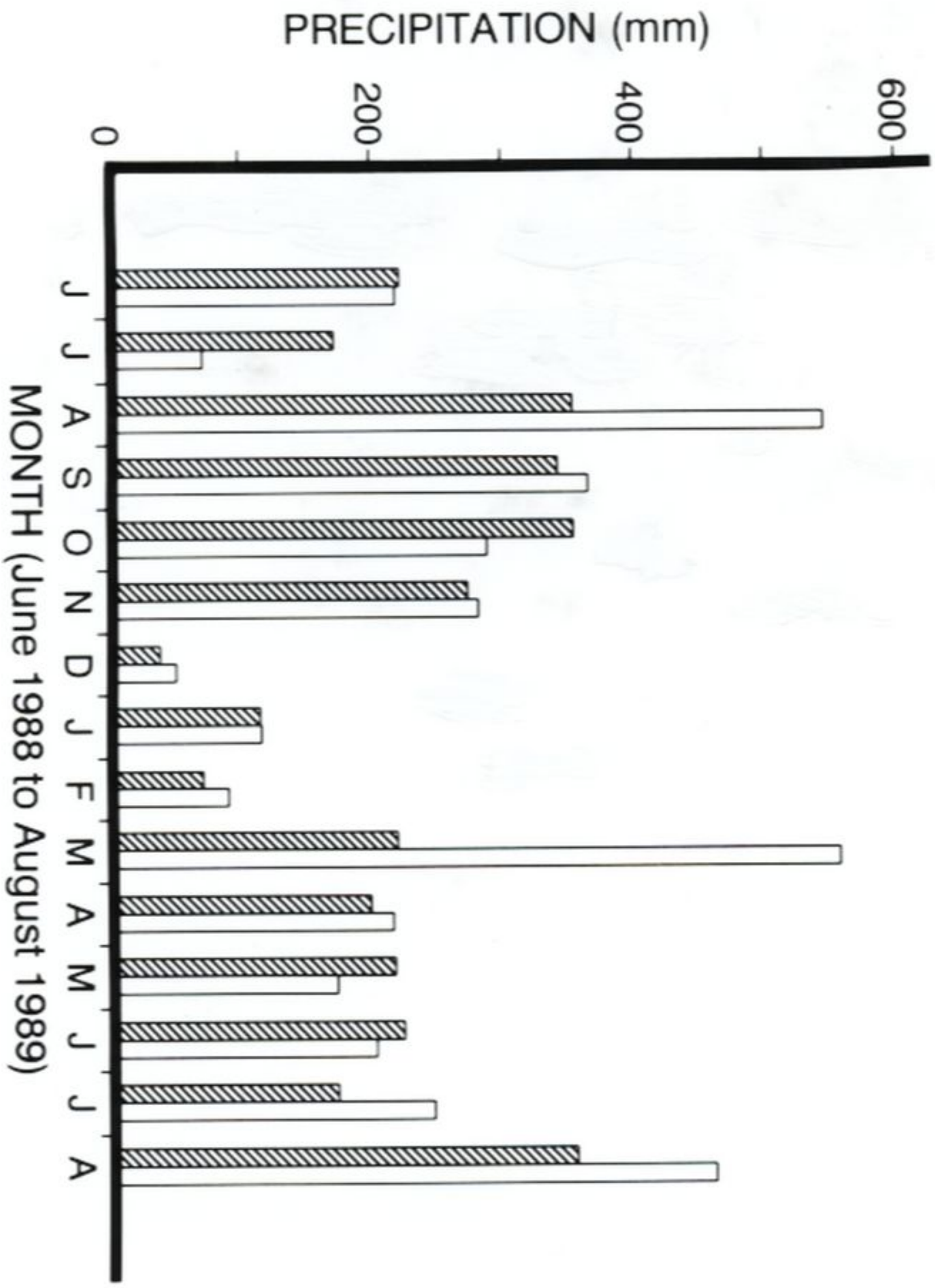

Fig. 1. Precipitation in the Tetas de Cerro Gordo sector. Empty bars are mean monthly precipitation during the research period. Shaded bars are mean monthly precipitation averaged over the years 1986-1990.

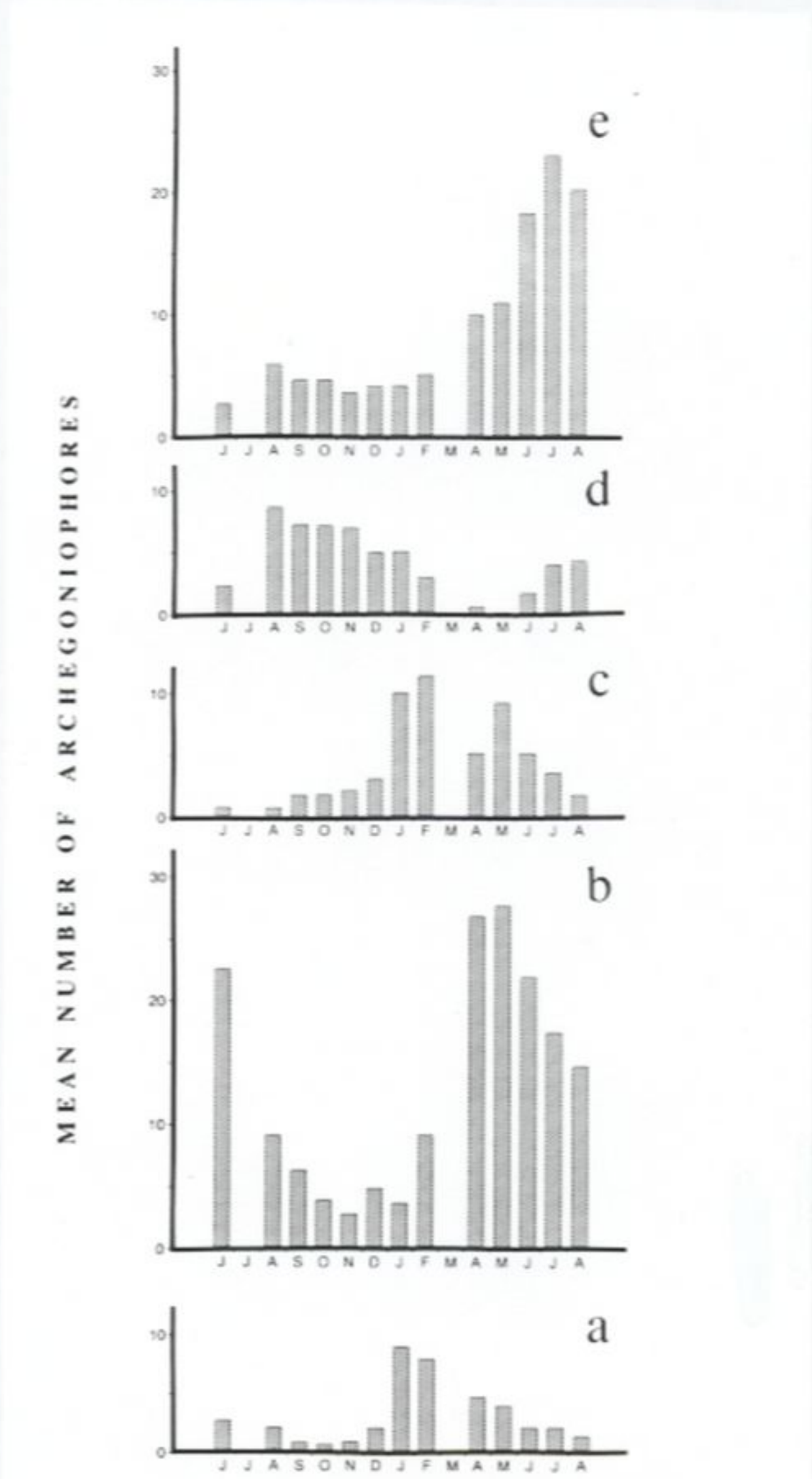

Fig. 2. Frequency of receptacles with archegonia (**a**), carpocephala with young sporophytes (**b**), carpocephala with mature sporophytes (**c**), carpocephala with sporophytes with open capsules (**d**), and aborted archegoniophores (**e**). Data for July 1988 and March 1989 were not collected.

throughout the population. The minimum distance between an antheridiophore and a sporophyte in the patches was 0.7 cm and the maximum distance was 65 cm. This agrees with the maximum dispersal distance of antherozoids stated by Parihar (1965) for the genus.

Newton (1971) found that the frequency of production of antheridia underestimates the actual number of male gametophytes in *Mnium undulatum*. It is possible that males were overlooked, were sterile or had disappeared from the population when censuses were made. This could explain the sporophytes scattered throughout the population of *M. chenopoda* with no antheridiophores near them. Antherozoid dispersal by insects attracted by the mucilage exuded by the paraphyses surrounding gametangia was observed in *Polytrichum commune* (Clayton-Greene et al., 1977). The possibility that animals, particularly insects, disperse the antherozoids cannot be totally discarded, and the role of water as a dispersal agent is yet to be investigated in *M. cenopoda*.

Carpocephala with young sporophytes (stage **b**) were observed throughout the year, but peak production occurred in late spring and summer, from April to July (Fig. 2b). The peak observed in June 1988 corresponds to the young sporophytes of the previous year. Different stages of development were observed when dissections were made. In general, the more developed sporophytes were located in the periphery of the carpocephalum while archegonia with unfertilized eggs were observed in the inner part. Fertilization of late-formed archegonia probably takes place after elevation of archegonial disks above the surface of the thallus (Parihar, 1965). According to Scagel et al. (1982), there exists a hormonal inhibition from the most advanced embryonic stages repressing the development of adjacent embryos. This may have a selective advantage because it promotes outcrossing for a longer period of time, and lowers resource competition among the developing zygotes. Regression analysis between rainfall and carpocephala with young sporophytes was not significant ($r^2 = 0.08$, $P > 0.05$).

Sporophyte maturation (stage **c**) occurred mainly during the dry season, from January to February, although another peak was observed in May (Fig. 2c). Regression analysis between rainfall and this stage was significant ($r^2 = 0.41$, $P < 0.05$). It seems that a decrease in moisture is necessary for the final maturation of the capsule.

Although spore release seemed to occur mainly during the rainy season, from August to November (Fig. 2d), regression analysis between rainfall and carpocephala with open capsules was not significant ($r^2 = 0.19$, $P > 0.05$). Protonemata were observed in the periphery of the population and in exposed muddy sites 500 m below the population. This seems to indicate that spore dispersal is effective, at least in this population. Spores of *M. chenopoda* were resistant to dessication and dry-freezing when exposed to upper atmosphere conditions (van Zanten & Gradstein 1987).

Most young sporophytes aborted by June to August (Fig. 2e). Abnormal rainfall was observed during March (Fig. 1); moderate to heavy rainfall occurred almost daily, producing runoff in varying quantities over the slope on which the population of *M. cenopoda* was located. Runoff may damage the archegoniophores and contribute to the high abortion rate observed. Regression analysis between rainfall and aborted archegoniophores was not significant ($r^2 = 0.01$, $P > 0.05$), but the possibility of selective abortion should not be discarded.

Gemmae cups were abundant throughout the year (Fig. 3). Gemmae cups with green gemmae (stage **a**) were mostly produced in April and May (Fig. 3a). Regression analysis between rainfall and this stage was not significant ($r^2 = 0.092$, $P > 0.05$). The gemmae matured and the cupules increased in diameter during the rainy season. The major production of dry, mature gemmae cups (stage **b**) was observed during the heaviest part of the rainy season, in September (Figure 3b). During this season, heavy rainfall occurs in the afternoon that easily could disperse the gemmae over longer distances. Regression analysis between rainfall and this stage was significant ($r^2 = 0.35$, $P < 0.05$).
It seems that a high proportion of bryophytes

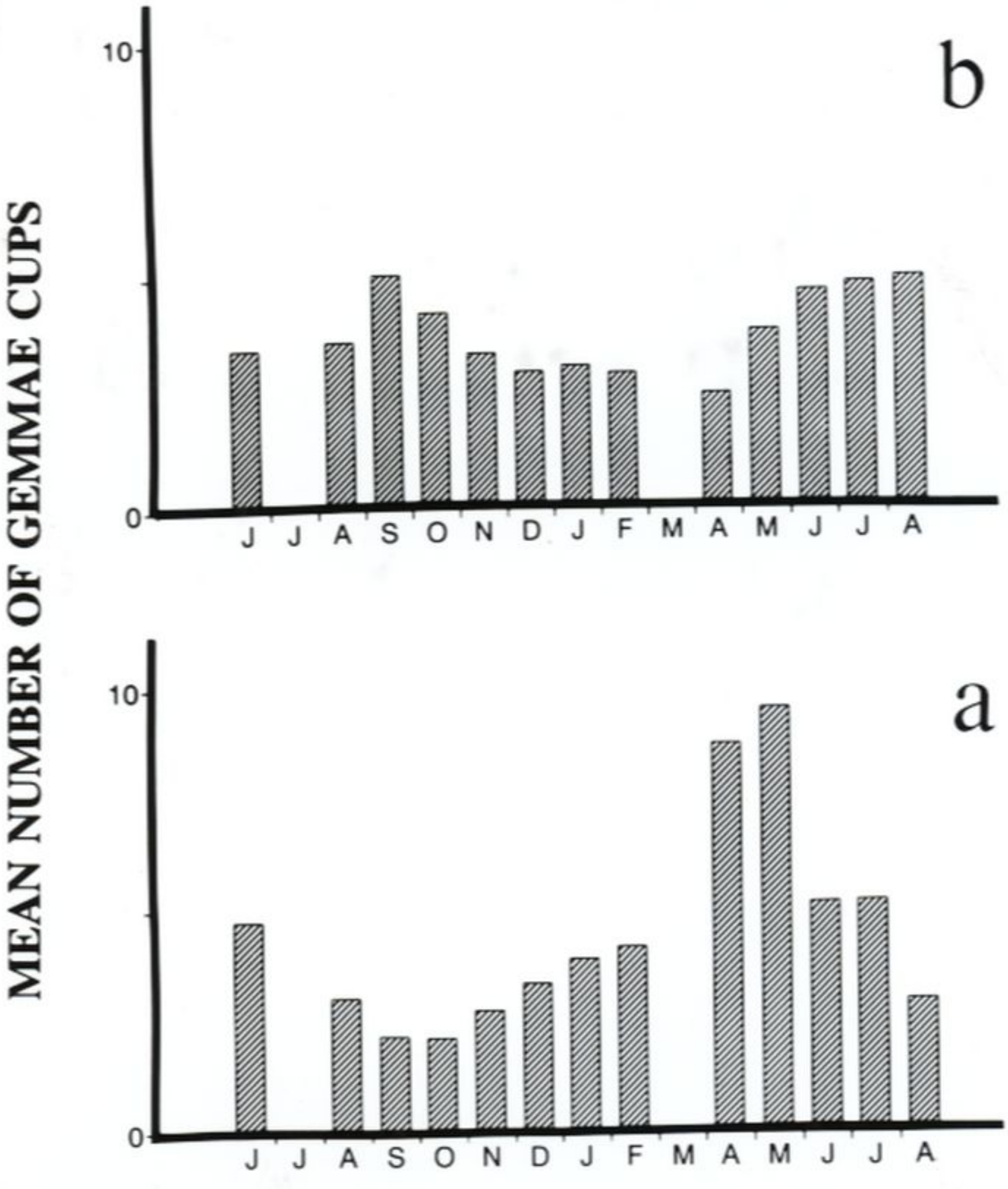

Fig. 3. Frequency of green gemmae cups (**a**), and dry gemmae cups (**b**). Data for July 1988 and March 1989 were not collected.

which rarely fruit are dioicous and that spatial separation of sexes, often associated with a rarity of plants bearing antheridia, may be the most general explanation of a rarity of sporophytes among mosses (Longton 1976). Schuster (1966) noted that sporophyte production occurs freely in many Marchantiales and more generally among hepatics of areas with wet climates as, for example, in the tropics. Several authors have commented on a correlation between the sexual habit of moss species and the frequency with which they produce sporophytes (Riemann 1972; Gemmell 1950). In general, a monoicous species would produce more sporophytes than a dioicous one. Quantitative data for liverworts are lacking, but the same general correlation between rarity of fruiting and dioicous habit may be consistent (Schuster 1966). According to the data obtained in this study, abortion rate should be taken into consideration as a factor associated with failure in sporophyte production.

Asexual propagules may assist in population expansion and gene flow, but should be potentially less effective than spores, at least in outbreeding taxa. It is thought that the general inefficiency of sexual reproduction among bryophytes may lead to reduced levels of genetic variation (Khanna 1964; Crum 1972). Data on genetic variation for this species are lacking. Research is now being conducted to determine the amount of genetic variation in the populations of *M. chenopoda* in Puerto Rico so that it may be compared with similar data for vascular plants.

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